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Letter to the Editor

Investigation of the Species Origin of the St. Jude Porcine Lung Epithelial Cell Line (SJPL) Made Available to Researchers▽

The SJPL cell line was reported and described as an immortalized porcine lung epithelial cell line suitable for influenza virus replication by Seo et al. (5) and has been available to researchers through ATCC (American Type Culture Collection) cell depositary services (deposit date, 5 April 2001; assigned accession number, PTA-3256) following a material transfer agreement. Recent findings indicate that the SJPL cell line is not of porcine origin but is more likely of monkey origin.

The percentage of acrosomic and telocentric chromosomes of the SJPL cell line obtained from ATCC was determined, and the findings were compared to what would be expected for a normal pig karyotype, as well as for a normal green monkey karyotype (Table 1). The domestic pig karyotype consists of 19 pairs of chromosomes (38 chromosomes in total), including 6 pairs of telocentric chromosomes and 2 pairs of chromosomes that can be classified as acrocentric (3). The African green monkey karyotype consists of 30 pairs of chromosomes (60 chromosomes in total), including 10 pairs of acrocentric chromosomes and no telocentric chromosomes (2). Acrocentric refers to the chromosomal configuration wherein the centromere is situated very close to one end of the chromosome, such that the short arm (p) is very small but still present, while telocentric refers to a chromosome configuration wherein the centromere is located at the terminal end of the chromosome, such that there is no short arm. Following karyotype analysis of SJPL cells obtained from ATCC, an absence of the characteristic porcine telocentric chromosomes, and a higher-than-expected ratio of acrocentric chromosomes per metaphase spread (25.5% for SJPL cells compared to 10.5% for porcine cells) were observed (Table 1). Overall, the acrocentric and telocentric chromosome ratios were more closely related to the monkey karyotype than to the porcine karyotype (Table 1). Furthermore, similar calculated acrocentric and telocentric chromosome ratios of the SJPL cells could be obtained after reevaluation of a chromosome analysis performed at the Core Cytogenetics Laboratory of St. Jude Children's Research Hospital in 2002 (Table 1). Consequently, the obtained results raise questions concerning the porcine origin of the SJPL cells.

To further determine the species origin of the SJPL cell line, primers were designed to amplify by PCR a portion of the P53 gene to verify the genomic DNA sequences of the SJPL cells compared to genomic DNA sequences of other cell types such as PK15 cells (porcine origin) and MARC-145 cells (African green monkey origin). As shown in Table 2, the P53 genomic sequence of the SJPL cells obtained from ATCC was more related to the MARC-145 monkey cell and human nucleotide sequences (100% and 95.2% nucleotide identities, respectively) compared to PK15 cells and pig nucleotide sequences (78.8% nucleotide identity for both). Based on chromosome analyses and genomic sequence analyses, the SJPL cells received from ATCC are not of pig origin but are more genomically related to monkey cells.

Following the first report of the SJPL cells (5), other investigators have used these cells in their studies (1, 4, 6, 7; C. A. Gagnon and M. Jacques, 9 December 2008, international patent application PCT/CA2008/001953). In some of those reports, the SJPL cells were used as a model to study viral and bacterial pathogenesis in regard to the respiratory tract environment of the susceptible host (1, 7). If the species origin of the SJPL cells is doubtful, then it is reasonable to question the respiratory tract origin of these cells. It is impossible for us to determine if the SJPL cells used in each of the published studies were obtained from ATCC or from another source and which cell passages were used and consequently to assume that they are monkey cells. Nonetheless, karyotype analyses of the SJPL cells originating from ATCC and from St. Jude Children's Research Hospital have both demonstrated the monkey origin of the cells (Table 1). Thus, in light of this recent finding, conclusions that have been drawn from previous studies using the SJPL cells should be carefully reconsidered.

Karyotyping. Chromosome analysis was performed on SJPL cells using standard methods. Briefly, SJPL cells were seeded onto petri dishes (60 by 15 mm) and grown under standard conditions. Mitotic arrest was induced by adding 150 μl colcemid (KaryoMax solution; Gibco) and incubating the cells for 30 min at 37°C. Cells were then trypsinized and centrifuged for 10 min at 1,000 rpm. A hypotonic shock was done by adding 10 ml KCl (0.075 M) to the cell pellet and by incubating the cell suspensions for 15 min at 37°C. Cell nuclei were pelleted and fixed by adding 10 ml of freshly made Carnoys's solution (methanol/glacial acetic acid, 3:1, vol/vol). The nuclear suspension was dropped onto microscope slides, which were allowed to dry and mature for several days. Afterward, slides were stained with KaryoMax Giemsa stain (Gibco). Slides were read for metaphase spreads using a Nikon Eclipse E800 microscope equipped with a Retiga 1300 (Q-Imaging) charge-coupled device camera and Simple PCI software for image analysis.

Sequence analysis. Genomic DNA extraction of cell lines was performed using the Qiagen QIAamp DNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. PCR primers were designed to target exon 8, intron 8-9,

TABLE 1. Comparison of acrocentric and telocentric chromosomes in the pig and green monkey genomes and SJPL cell line metaphase spreads

Cell origin	Total no. of chromosomes (2n)	% Acrocentric[e]	% Telocentric[e]
Sus scrofa (pig)	38	10.5 (2/19)	32 (6/19)
Cercopithews aethiops (green monkey)	60	33 (10/30)	0 (0/30)
SJPL[a]	Variable	25.5[b]	0
SJPL (2002)[c]	Variable	31[d]	0

[a] SJPL cell line obtained from ATCC and maintained at the Faculté de médecine vétérinaire, Université de Montréal.
[b] Average of eight analyzed metaphases.
[c] Data generated from a chromosome analysis performed at the St. Jude Children's Research Hospital in 2002.
[d] Average of three analyzed metaphases.
[e] The values in parentheses are chromosome type ratios.

TABLE 2. Partial P53 genomic sequence identities of SJPL cells with cells of porcine (PK15) and monkey (MARC-145) origins[a]

Cell type	% Identity			
	SJPL	PK15	MARC-145	Human[b]
PK15	78.8			
MARC-145	100	80.0		
Human[b]	95.2	79.9	95.2	
Pig[c]	78.8	98.1	78.8	82.4

[a] Base pair sequences of 209 nucleotides including most of exon 8, all of intron 8-9, and most of exon 9 were used for the analysis.
[b] Human TP53 sequences taken from Ensembl GRCh37 (February 2009).
[c] Pig P53 sequences taken from Ensembl Sscrofa9 (April 2009).

and exon 9 of the TP53 gene (P53 gene in humans). Based on sequence identity between the pig and human sequences, the sense primer p53.A (5′-GGACGGAACAGCTTTGAGGT GCG) and the degenerate reverse primer p53.1 (5′-AGGGT GAAATA[T/C]TCICCATCCAGTG, where I is inosine) were designed and synthesized (Invitrogen). From pig genomic DNA, these primers should amplify a band of 307 bp; from human genomic DNA, they should amplify a band of 303 bp. The extracted DNA was amplified by PCR with *Taq* DNA polymerase (New England Biolabs, Ipswich, MA). The PCR consisted of an initial enzyme activation step at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 45 s, extension at 72°C for 1 min 30 s, and a final extension at 72°C for 5 min. The PCR products were purified using a commercial kit (QIAquick PCR purification kit; Qiagen) according to the manufacturer's instructions. Sequencing reactions of amplified bands were performed using the dideoxy method and Big Dye Terminator 3.1 (Applied Biosystems) reagents according to the manufacturer's instructions. The same PCR primers were used for the sequencing reactions. Sequencing was performed on an ABI Prism 310 genetic analyzer. Identity comparisons were performed using MacDNAsis software (Hitachi).

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